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Ampicillin Susceptibility Can Predict *In Vitro* Susceptibility of Penicillin-Resistant, Ampicillin-Susceptible *Enterococcus faecalis* Isolates to Amoxicillin but Not to Imipenem and Piperacillin

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Our findings demonstrated that the results obtained for ampicillin may accurately predict the *in vitro* susceptibility to amoxicillin but not to imipenem and piperacillin among isolates of *Enterococcus faecalis* resistant to penicillin but susceptible to ampicillin, which have emerged recently, in contrast to penicillin- and ampicillin-susceptible isolates.

Enterococci are intrinsically resistant to several antimicrobial classes and show a great ability to acquire new mechanisms of resistance. Resistance to β-lactam antibiotics is a great concern because these drugs are commonly used for treatment of enterococcal infections, alone or associated with aminoglycosides, since such combination therapy results in the synergistic killing of the enterococci (5). β-Lactamase production, overproduction of low-affinity penicillin-binding proteins (PBPs), and occurrence of point mutations in PBPs, especially PBP5, are the mechanisms of β-lactam resistance that have been reported in enterococci (5, 12).

Although all enterococci are intrinsically resistant to cephalosporins, *Enterococcus faecalis* remains usually susceptible to the other β-lactam antibiotics, including the carbapenems, in contrast to *Enterococcus faecium*. Furthermore, until recently, it was assumed that *E. faecalis* strains exhibiting susceptibility to ampicillin were also susceptible to penicillin; however, the emergence of isolates resistant to penicillin but susceptible to ampicillin showed that the resistance to both β-lactams may not be linked in enterococci (6, 8). Currently, according to the Clinical and Laboratory Standards Institute (CLSI) (3), ampicillin results may be used to predict *E. faecalis* susceptibility to amoxicillin, imipenem, and piperacillin among non-β-lactamase-producing *E. faecalis* strains, while isolates susceptible to ampicillin cannot be assumed to be susceptible to penicillin. Therefore, as there are few published studies about penicillin-resistant, ampicillin-susceptible *E. faecalis* strains, we propose here to evaluate whether the susceptibility to ampicillin can really predict the susceptibility to amoxicillin, imipenem, and piperacillin among *E. faecalis* isolates exhibiting this unusual penicillin resistance phenotype.

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A collection of 317 *E. faecalis* isolates, recovered during a study conducted at a Brazilian hospital in the period of February 2006 to June 2010 (4), was tested for ampicillin and penicillin susceptibility. Thirty-four (10.7%) isolates were penicillin resistant and ampicillin susceptible in the three susceptibility tests performed (Etest, broth dilution, and disk diffusion). The species identification of all selected isolates was performed based on phenotypic tests (9) and confirmed by PCR using specific primers described elsewhere (4). These isolates were recovered from wounds (35.3%), urine (32.4%), secretions (14.7%), blood (11.8%), and catheter tip (5.9%). They showed a MIC range of 1 to 8 μg/ml for ampicillin and 16 to 32 μg/ml for penicillin. In addition, 15 randomly selected isolates of *E. faecalis* susceptible (MIC, ≤8 μg/ml) simultaneously to penicillin and ampicillin were also evaluated for comparative purposes. All selected isolates were susceptible to vancomycin; 26.7% (4/15) of penicillin-susceptible, ampicillin-susceptible isolates and most (79.4%, 27/34) of the penicillin-resistant, ampicillin-susceptible isolates were resistant to high levels of gentamicin. Ethics approval was obtained for this study.

TABLE 1 Comparison of rates of susceptibility to amoxicillin, imipenem, and piperacillin obtained by different tests among penicillin-resistant, ampicillin-susceptible *Enterococcus faecalis* isolates ($n = 34$)

β-Lactam antibiotic	No. (%) of susceptible isolates by test:			*P* value
	Disk diffusion	Broth dilution	Etest	
Amoxicillin	31 (91.2)	34 (100)	34 (100)	0.05
Imipenem	13 (38.2)	25 (73.5)	21 (61.8)	0.011
Piperacillin	9 (26.5)	0 (0)	0 (0)	<0.001

Ampicillin (10-μg), penicillin (10-U), amoxicillin (10-μg), imipenem (10-μg), and piperacillin (100-μg) disks (Oxoid, England) and Mueller-Hinton agar (Difco, Becton, Dickinson and Company, Sparks, MD) were used for disk diffusion testing. The MIC was determined by Etest (AB bioMérieux, Solna, Sweden) and by the broth dilution method that was performed using Mueller-Hinton broth (Difco, Becton, Dickinson and Company, Sparks, MD) and solutions of antimicrobials prepared from powders of known potencies (Sigma-Aldrich Denmark A/S, Copenhagen, Denmark). Susceptibility tests were performed and interpreted according to the CLSI (1, 2, 3) guidelines. For all the β-lactams tested, MICs of ≥16 μg/ml indicated resistance. Current quality control

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TABLE 2 β-Lactam MIC ranges and MIC_{90}s for groups of penicillin-resistant, ampicillin-susceptible *Enterococcus faecalis* ($n = 34$) and penicillin- and ampicillin-susceptible *E. faecalis* ($n = 15$) isolates by broth dilution and Etest

β-Lactam antibiotic	Test[a]	Group of *E. faecalis* isolates[b]	Cumulative no. (%) of isolates inhibited at MIC (μg/ml):									MIC_{90} (μg/ml)
			≤0.5	1	2	4	8	16	32	64	≥128	
Ampicillin	BD	Pen-S		11 (73.3)	15 (100)							2
		Pen-R		1 (2.9)	4 (11.7)	23 (67.6)	34 (100)					8
	Etest	Pen-S	10 (66.6)	15 (100)								1
		Pen-R			13 (38.2)	33 (97.0)	34 (100)					4
Amoxicillin	BD	Pen-S	12 (80.0)	14 (93.3)	15 (100)							1
		Pen-R	3 (8.8)	9 (26.4)	23 (67.6)	33 (97.0)	34 (100)					4
	Etest	Pen-S	14 (93.3)	15 (100)								0.5
		Pen-R	4 (11.6)	22 (64.6)	29 (85.2)	34 (100)						4
Imipenem	BD	Pen-S	2 (13.3)	14 (93.3)	15 (100)							1
		Pen-R					25 (73.5)	34 (100)				16
	Etest	Pen-S	13 (86.7)	15 (100)								1
		Pen-R				3 (8.8)	21 (61.7)	27 (79.3)	34 (100)			32
Piperacillin	BD	Pen-S			4 (26.7)	11 (73.4)	15 (100)					8
		Pen-R							2 (5.9)	12 (35.3)	34 (100)	≥128
	Etest	Pen-S		10 (66.7)	13 (86.7)	15 (100)						4
		Pen-R								4 (11.8)	34 (100)	≥128

[a] BD, broth dilution.
[b] Pen-S, group of penicillin- and ampicillin-susceptible *E. faecalis* isolates; Pen-R, group of penicillin-resistant, ampicillin-susceptible *E. faecalis* isolates.

testing was performed using the following organisms: *Staphylococcus aureus* ATCC (American Type Culture Collection) 25923, *Escherichia coli* ATCC 25922, and *E. faecalis* ATCC 29212.

β-Lactamase production was tested with a chromogenic nitrocefin disk (Cefinase; Becton, Dickinson and Company), but none of the *E. faecalis* isolates included in this study produced β-lactamase. *S. aureus* ATCC 29213 was used as a positive control.

The chi-square or Fisher exact test was used to compare the susceptibility rates. Differences were considered significant at P values of <0.05.

As demonstrated in Table 1, all (100%) of the penicillin-resistant, ampicillin-susceptible *E. faecalis* isolates evaluated were susceptible to amoxicillin by Etest and broth dilution, while 91.2% were susceptible by disk diffusion, indicating a great correlation between amoxicillin and ampicillin results. On the other hand, a lower correlation was observed between imipenem and ampicillin susceptibilities using disk diffusion (38.2%), Etest (61.8%), and broth dilution (73.5%). Piperacillin was the β-lactam that showed the poorest correlation with ampicillin. There were no significant differences in the results obtained by Etest and broth dilution for all β-lactams tested, in contrast to those obtained by disk diffusion for imipenem and piperacillin. Among the penicillin- and ampicillin-susceptible *E. faecalis* isolates, the agreement rate between ampicillin and the other β-lactams evaluated was 100% in all testing (data not shown).

Table 2 summarizes the MIC range and MIC_{90} values obtained by Etest and broth dilution for the β-lactam antibiotics. Among the penicillin- and ampicillin-susceptible *E. faecalis* isolates, the MIC_{90} values ranged from 0.5 to 2 μg/ml for ampicillin, amoxicillin, and imipenem and from 4 to 8 μg/ml for piperacillin. Note that among the penicillin-resistant, ampicillin-susceptible *E. faecalis* isolates, the MIC_{90} values were slightly higher (4 to 8 μg/ml) for ampicillin and amoxicillin, although the values remained within the susceptibility range. For imipenem, the MIC_{90} values ranged from 16 to 32 μg/ml, and that for piperacillin was ≥128 μg/ml.

Taken together, our results suggest that ampicillin susceptibility can predict the *in vitro* susceptibility of penicillin-resistant, ampicillin-susceptible *E. faecalis* isolates to amoxicillin but not to imipenem and piperacillin. Conversely, other studies have shown that ampicillin is an accurate predictor of the *in vitro* activity of imipenem (10, 11) and piperacillin (7) against *E. faecalis*. Nevertheless, it is quite probable that penicillin-resistant, ampicillin-susceptible *E. faecalis* isolates had not been tested in those studies since this unusual penicillin resistance phenotype was first reported in 2005 (8).

Metzidie et al. (8) found in a Greek hospital a higher rate than ours (31.4% versus 10.7%) of penicillin-resistant, ampicillin-susceptible *E. faecalis* isolates, and most of them were also resistant to imipenem. Recently, Guardabassi et al. (6) reported the spread of penicillin-resistant, ampicillin-susceptible *E. faecalis* isolates among bloodstream infections in Denmark, which were susceptible to vancomycin and mostly were resistant to gentamicin, similar to the isolates of the present study.

In conclusion, our findings demonstrated that ampicillin susceptibility should not be used to predict susceptibility to imipenem and piperacillin among *E. faecalis* isolates that have acquired this unusual penicillin resistance phenotype, as well as showing the need for changes in the current CLSI as well as EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines for susceptibility testing of this group of *E. faecalis* isolates. However, as we evaluated a relatively small number of isolates and they were from a single institution, further studies using penicillin-resistant, ampicillin-susceptible *E. faecalis* isolates from other geographic regions are warranted to confirm our results and to clarify the mechanism of resistance displayed by these isolates.

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We declare that there is no conflict of interest.

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